

EXPERT OPINION

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Preclinical discovery of duloxetine for the treatment of depression

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Introduction: Affective disorders, including major depressive disorder (MDD), are among the most severely disabling mental disorders, and in many cases are associated with poor treatment outcomes. From the emergence of the monoamine hypothesis of depression, the first-line treatment for MDD had mainly acted by inhibiting monoamine reuptake, and thereby increasing these levels in the synaptic cleft. However, in recent years, several new antidepressant drugs have appeared, including duloxetine, a dual serotonin (5-HT) and noradrenaline (NA) reuptake inhibitor recommended for the treatment of MDD.

Areas covered: The article reviews and discusses the biochemical and functional profile of duloxetine splitting the review into acute and long-term treatment with this dual monoamine reuptake inhibitor. In addition, the authors summarize available preclinical behavioral research data, which have demonstrated among other effects, the antidepressant-like activity of duloxetine in several animal models. The authors focus on the most recent literature on synaptic neuroplasticity modulation of this antidepressant drug. Finally, the authors briefly mention other approved indications of duloxetine.

Expert opinion: Duloxetine inhibits 5-HT and NA reuptake, effectively desensitizes various autoreceptors and promotes neuroplasticity. Clinically, duloxetine is an effective antidepressant that is well tolerated and has significant efficacy in the treatment of MDD.

Keywords: depression, dual-action antidepressant, duloxetine, noradrenaline, serotonin

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1. Introduction

Affective disorders, which include major depressive disorder (MDD), are among the most prevalent forms of mental illness and it is estimated that 15% of the population will suffer a depressive episode at some point in their lifetime [1,2]. According to the World Health Organization, depression is one of the top 10 causes of morbidity and mortality worldwide, and it is expected to be the second most common cause of disability by 2020 [3]. Given the high prevalence of this disorder, its frequently chronic nature and the associated risk of suicide, the development of new therapeutic strategies to treat this condition is paramount.

Antidepressant treatments were first introduced in the 1950s, and the first drugs used to treat depression were tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOI). The monoamine hypothesis of depression [4] was introduced in the following decade, which maintained that deficits in brain serotonin (5-HT) and noradrenaline (NA) gave rise to depression. Accordingly, in the 1970s and 1980s neuropsychopharmacology focused on the development of drugs that selectively increased the levels of these monoamines. These drugs, now known as selective serotonin reuptake inhibitors (SSRIs) and noradrenaline reuptake inhibitors (NRIs), inhibit the uptake of catecholamines and promote their bioavailability at the synaptic cleft. Subsequent attempts to develop new antidepressant compounds that enhance

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Article highlights.

- There is an urgent need for new compounds to treat major depressive disorder (MDD).
- Duloxetine is a dual serotonin (5-HT) and noradrenaline (NA) reuptake inhibitor, with high affinity for 5-HT transporter (SERT) and NA transporter (NET), and weak affinity for the dopamine (DA) transporter.
- Chronic duloxetine treatment induces adaptive changes in the 5-HT and NA systems, including autoreceptor desensitization.
- Animal behavioral studies have demonstrated the antidepressant- and anxiolytic-like effects of duloxetine, in the absence of cognitive impairment.
- Duloxetine modulates neuronal plasticity by enhancing the expression of neurotrophic factors such as brain-derived neurotrophic factor (BDNF).
- Duloxetine alleviates the physical symptoms of depression, including aches and pains.

This box summarizes key points contained in the article.

monoaminergic (i.e., both serotonergic and noradrenergic) neurotransmission led to the synthesis of dual monoamine reuptake inhibitors, such as duloxetine.

Duloxetine was created by researchers at Eli Lilly and it was first described in its racemic form (LY227942) in 1988. Subsequently, the most potent enantiomer (LY248686 or duloxetine) [5] was approved by the US Food and Drugs Administration (FDA) for the treatment of major depression.

2. Duloxetine: biochemical and functional profile

Duloxetine (LY248686, *N*-methyl-*g*-1-naphthalenyloxy-2-thiophene propanamine hydrochloride; **Figure 1**) is a dual inhibitor of 5-HT and NA reuptake. The binding profile of duloxetine in rat cortical synaptosomes demonstrates its inhibition of the 5-HT transporter (SERT) and NA transporter (NET), with K_i values of 0.5 and 3.6, respectively [6,7]. These values are in agreement with the results of *ex vivo* binding assays performed in hippocampal and cortical slices [6,8]. Duloxetine thus inhibits 5-HT transport with greater potency than NA transport (**Table 1**), with little or no affinity for other neuronal receptors [9].

Duloxetine inhibits 5-HT and NA uptake in hypothalamic and cortical synaptosomes, as evident through *in vitro* uptake assays [7,10,11], with only a weak effect on dopamine (DA) uptake in striatal synaptosomes [7] (**Table 1**). In *ex vivo* uptake assays, acute duloxetine treatment inhibits both 5-HT and NA reuptake in the rat hippocampus and hypothalamus [7,8].

In fact, duloxetine presents a higher affinity for both 5-HT and NA transporters than TCAs [12]. In addition, duloxetine has a low selectivity ratio for NA/5-HT reuptake inhibition [6]. Thus, compared with venlafaxine, as other dual antidepressant, duloxetine is the most balanced dual transporter inhibitor (**Table 1**) [6,12].

2.1 Acute duloxetine treatment

In rat microdialysis studies, acute duloxetine increases 5-HT, NA and DA levels in the cerebral cortex [13-17], 5-HT and NA levels in the hippocampus [11,18] and DA levels in nucleus accumbens [15] in a dose-dependent manner. The nucleus accumbens plays a critical role in the reward pathway and is implicated in the pathophysiology of depression [19,20]. The increase in DA levels in this region may reflect the affinity of duloxetine for the DA transporter, although very high concentrations of duloxetine are required to inhibit DA uptake. Thus, the increases in DA observed are more likely an indirect effect of the increases in 5-HT and NA levels. Indeed, increased DA has been reported in the prefrontal cortex following NRI treatment [21,22].

By inhibiting 5-HT and NA reuptake, duloxetine reverses the depletion of 5-HT induced by p-CA ((±) *para*-chloroamphetamine) and the depletion of NA by 6-OHDA (6-hydroxydopamine hydrobromide) or α -MMT (α -methyl-*m*-tyrosine) administration [6,16,23,24] in a dose-dependent manner. Interestingly, duloxetine reverses MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced NA depletion in the cerebral cortex but not the striatum [23], further supporting the view that DA levels are modulated indirectly by duloxetine-induced increases in 5-HT and NA levels.

In several brain regions implicated in the therapeutic effect of antidepressants, duloxetine reduces the 5-HT turnover rate. This is determined by measuring the ratio of 5-HT to its principal metabolite (5-hydroxyindoleacetic acid (5-HIAA)), which has been correlated with an increase in 5-HT release into synaptic clefts [23,25].

The effects of duloxetine have been analyzed when administered in combination with ligands of autoreceptors that modulate serotonergic and noradrenergic activity. 5-HT_{1A} autoreceptors and α_2 -adrenoreceptors negatively regulate the release of 5-HT and NA in serotonergic and noradrenergic neurons, respectively. Antagonism of 5-HT_{1A} with WAY100,635 or LY206130 potentiates the duloxetine-induced increase in 5-HT but not that of NA or DA in the rat frontal cortex and hypothalamus [17,26]. By contrast, co-administration of the 5-HT_{1A} agonist buspirone decreases cortical 5-HT levels, while augmenting the NA and DA in this structure. These increases in NA and DA may reflect the antagonistic properties of buspirone at inhibitory autoreceptors in the noradrenergic and dopaminergic systems [27]. In the presence of duloxetine, the α_2 -adrenoceptor antagonists atipamezole and I-PP (1-(2-pyrimidyl)piperazine) potentiate the accumulation of 5-HT, NA and DA in the rat frontal cortex [14]. By contrast, the effects of duloxetine are unaltered by the β -adrenergic antagonist metoprolol [26].

In summary, acute duloxetine administration in rats induces a general increase in catecholamine levels, and it reverses pharmacological depletion of 5-HT and NA. Moreover, the 5-HT_{1A} autoreceptor and the α_2 -adrenoreceptor play essential roles in the mode of action of duloxetine.

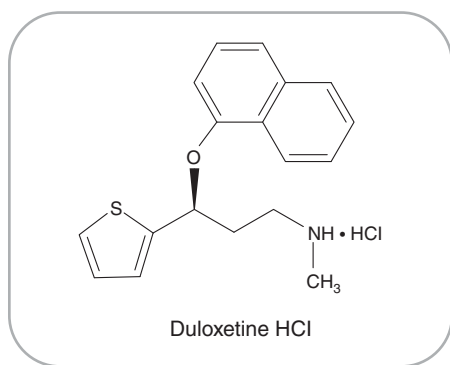


Figure 1. Chemical structure of duloxetine hydrochloride.

Table 1. *In vitro* binding affinity of duloxetine for 5-HT, NA and DA transporters, and for the inhibition of catecholamine reuptake and antidepressants *in vitro* binding affinity for 5-HT and NA human transporters.

Binding affinity and inhibition uptake	K _i (nM)
[³ H]-Paroxetine <i>in vitro</i> binding [7]	0.5 ± 0.1
[³ H]-Nisoxetine <i>in vitro</i> binding [6]	3.6 ± 0.3
[³ H]-5-HT uptake in cortical snps [7]	4.6 ± 1.1
[³ H]-NA uptake in hypothalamic snps [7]	15.6 ± 2.9
[³ H]-DA uptake in striatal snps [7]	369.2 ± 38.1
[³ H]-5-HT uptake in whole brain snps [11]	1.9
[³ H]-NA uptake in cortical snps [11]	3.1

Human antidepressants profile	SERT	NET
Duloxetine [6]	0.80 ± 0.04	7.50 ± 0.30
Venlafaxine [12]	8.90 ± 0.30	1060 ± 40
Amitriptyline [12]	4.30 ± 0.12	35 ± 2
Nortriptyline [12]	18 ± 1	4.37 ± 0.07
Imipramine [12]	1.40 ± 0.03	37 ± 2
Fluoxetine [12]	0.81 ± 0.02	240 ± 10
Citalopram [12]	1.16 ± 0.01	4070 ± 80
Paroxetine [12]	0.13 ± 0.01	40 ± 2
Sertraline [12]	0.29 ± 0.01	420 ± 20
Mirtazapine [12]	>100000	4600 ± 300

K_i: Mean of affinity constants expressed in nM ± S.E.M. (Standard error of the mean); 5-HT: Serotonin; NA: Noradrenaline; DA: Dopamine; snps: Synaptosomes; NET: Noradrenaline transporter; SERT: Serotonin transporter.

2.2 Long-term duloxetine treatment

Chronic duloxetine treatment exerts a long-term modulatory effect on serotonergic and noradrenergic systems. *In vivo* microdialysis studies revealed that chronic duloxetine treatment has no effect on basal levels of 5-HT, NA or DA in the rat frontal cortex [15]. Accordingly, chronic treatment with SSRIs or NRIs fails to modify 5-HT and NA levels, respectively [28,29]. These findings suggest that, the mode of action of duloxetine when administered chronically involves adaptive changes that alter autoreceptor function, as previously described following long-term SSRI treatment [30-32].

In *ex vivo* uptake and binding studies in rodents, chronic duloxetine treatment reduces the density of functional SERT in the cortex [6,33-35], although no increase in 5-HT release is produced in this brain region [34]. Strikingly, although 5-HT transport is deregulated in the cortex, chronic duloxetine treatment does not increase the release of this monoamine. However, several studies have described increased 5-HT release in response to the SERT down-regulation that follows chronic SSRI treatment [36-38]. This discrepancy between the effects of chronic duloxetine and SSRI regimens remains unexplained, although it is possible that the noradrenergic effects of duloxetine underlie these differences. Alternatively, the increases in NA observed in the cortex may be directly due to a reduction in the functional NET induced by duloxetine in this brain area [6,34]. Further research will be necessary to elucidate the mechanisms that underlie this effect.

In *ex vivo* uptake assays, chronic duloxetine treatment augments 5-HT release in the hippocampus following, although it has no effect on the density of functional SERT [34]. *In vivo* extracellular recordings of CA3 hippocampal neurons have demonstrated that the recovery time of the firing activity of these neurons following microiontophoretic 5-HT administration is similar after acute and chronic duloxetine treatment [8,39]. The recovery time provides a measure of the *in vivo* activity of monoamine reuptake systems [38,40] and thus, these results suggest that chronic duloxetine does not modify SERT function. Moreover, duloxetine has no effect on the inhibitory firing rate of hippocampal neurons, a parameter that reflects postsynaptic receptor sensitivity [41]. This finding indicates that chronic duloxetine treatment does not modify postsynaptic receptors in hippocampal neurons [39]. However, when a lower dose of duloxetine was used a decrease in SERT density was observed [33]. The different duloxetine doses used in various studies may explain the variations in the observed adaptive changes.

Following chronic duloxetine administration in rats, no alterations in functional SERT density were observed in the hippocampus, nor was 5-HT_{1B} autoreceptor sensitivity altered in serotonergic neurons [34], even though these autoreceptors are desensitized by chronic SSRI treatment [42]. One explanation for these different effects is the dual nature of duloxetine reuptake inhibition, which may desensitize α₂-heteroreceptors in 5-HT terminals [34], as previously described following chronic NRI and MAOI treatment [36,43].

The dorsal raphe nucleus and locus coeruleus are key brain regions involved in the modulation of serotonergic and noradrenergic transmission, respectively, each of which emits projections to a wide array of structures, including the cortex and hippocampus. Chronic SSRI treatment augments 5-HT release and desensitizes SERT in the dorsal raphe [38] and while duloxetine also enhances 5-HT release in this region, it has no effect on SERT activity in *ex vivo* uptake assays [34]. Altered presynaptic receptor function most probably explains these increases in 5-HT release and indeed, desensitization of somatodendritic 5-HT_{1A} and 5-HT_{1D} autoreceptors was described following chronic duloxetine treatment [34], which

in turn may negatively regulate 5-HT release [44]. Extracellular recordings from dorsal raphe neurons revealed a dose-dependent inhibition of the firing of these neurons following acute duloxetine administration [8,39,45], an effect that does not persist when this drug is received chronically [39]. Given that somatodendritic 5-HT_{1A} autoreceptors negatively control the spontaneous firing rate of dorsal raphe neurons [44], desensitization of these receptors following long-term duloxetine treatment may underlie the restoration of basal firing rates [39].

Chronic duloxetine treatment increases NA release in the hippocampus, while having no effect on the density or function of the NET or the β 1-noradrenergic receptor [6,34]. This increase in NA is a result of the desensitization of α ₂-heteroreceptors in NA terminals [34,39], consistent with the desensitization of α ₂-heteroreceptors observed after chronic NRI treatment [43,46]. *In vivo* extracellular recordings in CA3 hippocampal neurons have revealed that both acute and chronic duloxetine administration enhances the recovery time of the firing activity of these neurons following microiontophoretic NA administration [8,39], suggesting that chronic duloxetine does not modify NA transport. In addition, duloxetine has no effect on the inhibitory firing rate of hippocampal neurons, indicating that the sensitivity of postsynaptic receptors remains unaltered [39,41].

Based on the desensitization of α ₂-heteroreceptors in NA terminals observed, the function of somatodendritic α ₂-autoreceptors was evaluated through *in vivo* electrophysiological recordings of locus coeruleus neurons. Acute duloxetine inhibited the spontaneous electrical activity of these neurons in a dose-dependent manner [8], an effect that was reversed by the α ₂-adrenoceptor antagonist idazoxan. These findings demonstrate that somatodendritic α ₂-autoreceptors are not desensitized by duloxetine.

In summary, chronic duloxetine treatment has no effect on basal 5-HT, NA or DA levels in the cerebral cortex, although it enhances NA but not 5-HT release, and it induces SERT and NET dysfunction. In the hippocampus, chronic duloxetine augments 5-HT and NA release, yet it has no effect on SERT and NET density, while desensitizing terminal α ₂-heteroreceptors but not 5-HT_{1B} receptors. In the dorsal raphe nucleus, chronic duloxetine augments 5-HT release but it does not affect SERT density, while it desensitizes somatodendritic 5-HT_{1A} and 5-HT_{1D} autoreceptors. Finally, several studies have described crosstalk between the serotonergic and noradrenergic systems [8,47], which may have important implications for the mode of action of duloxetine.

3. Behavioral studies

Several preclinical studies of behavior also evaluated the biochemical and functional profile of duloxetine, analyzing its effects on locomotor activity, body weight, food intake, corporal temperature, ptosis, tremor, memory and sleep, in addition to its antidepressant and anxiolytic effects (Table 2).

In rodents, duloxetine treatment reduces food intake [48,49], while chronic treatment in high doses leads to a decrease in body weight [48,50], consistent with the decreased body weight reported in duloxetine-treated patients [51]. Duloxetine also reduces the body temperature [52], although it alleviates the hypothermia induced by reserpine, 8-OHDPAT (8-hydroxyl-2-(di-*n*-propylamino)tetralin and mCPP (*m*-chlorophenyl piperazine) [35,52]. Moreover, duloxetine reverses tetrabenazine-induced ptosis in a dose-dependent manner [52]. Duloxetine partially antagonizes oxotremorine-induced tremor in mice, although it provokes a dose-dependent increase in head movements and the tremor score induced by a precursor of 5-HT. In rats, this latter combination produced an increase in head movement but not in tremor [52].

Sleep abnormalities are one of the primary symptoms of major depressive disorder (Diagnostic and Statistical Manual IV Text Revision, DSMIV-TR) [20,53] and accordingly, several studies have analyzed the effect of duloxetine on sleep using rat electroencephalogram (EEG) recordings. A decrease in rapid eye movement (REM) and slow-wave deep sleep was reported following acute duloxetine treatment, along with a parallel increase in the awake period [52]. However, no such effects of duloxetine on REM and slow-wave deep sleep were observed in a subsequent study, despite the decreased paradoxical sleep and increased awake period produced [11]. The discrepancies between these studies may be explained by the different duloxetine doses employed. Notably, significant disruption of sleep architecture has been demonstrated following NRI and dual-action antidepressant treatment [11], suggesting that the noradrenergic properties of duloxetine may underlie its effects on sleep.

The antidepressant-like activity of duloxetine has been measured in the forced swimming test (FST), a commonly used test in which reduced immobility time is correlated with antidepressant activity [54,55]. Acute and subacute duloxetine treatment decreased the immobility time in the FST in most studies [52,56-60]. However, when a lower dose was used no such decrease in immobility time was observed after acute duloxetine administration, yet antidepressant-like effects were observed when duloxetine was co-administered with a 5-HT_{1A} antagonist (WAY100,635) [17]. The antidepressant-like effects of duloxetine were also enhanced when it was administered in combination with an AMPA receptor potentiator (LY392098) [58]. Moreover, duloxetine also induces antidepressant-like effects in the mouse tail suspension test (TST) [61]. As predictive tests of antidepressant activity measure active animal behavior, it is crucial to evaluate spontaneous locomotor activity to ensure the results are correctly interpreted and to avoid false positives. At the higher end of the dose range used in preclinical studies, antidepressants decrease spontaneous locomotor activity [60]. Similarly, higher subacute doses of duloxetine decrease locomotor activity [60], while acute and chronic treatment with lower doses has no such effect [24,35,62-64]. Importantly, no increase in locomotor activity has been reported following duloxetine administration at any dose, indicating that the observed effects in FST and TST are not due to the induction of hyperactivity.

Table 2. Published studies of duloxetine for preclinical behavioral tests.

	Duloxetine dose	Effect
Body weight (rat)	Chronic; 100 mg/kg p.o. (14 days)	↓Body weight [50]
	Chronic; 30 mg/kg p.o. (4 weeks)	↔ Body weight [48]
Food intake (rat)	Acute; 30 mg/kg p.o.	↓ Food intake [49]
	Chronic; 30 mg/kg p.o. (4 weeks)	↓ Food intake [48]
<i>Body temperature (mouse)</i>		
Reserpine-induced hypothermia	Acute; 3.13 – 12.5 mg/kg p.o.	↓ Hypothermia [35,52]
8-OHDPAT-induced hypothermia	Chronic; 10 mg/kg p.o. (twice daily, 28 days)	
mCPP-induced hypothermia	Chronic; 10 mg/kg p.o. (twice daily, 28 days)	
<i>Ptosis</i>		
Tetrabenazine-induced ptosis (mouse)	Acute; 3.13 – 25 mg/kg p.o.	↓ Ptosis (dose-dependent) [52]
Tetrabenazine-induced ptosis (rat)	Acute; 12.5 – 50 mg/kg p.o.	
<i>Tremor</i>		
5-HTP-induced tremor (mouse)	Acute; 25 – 100 mg/kg p.o.	↑Tremor score (dose-dependent) [52]
5-HTP-induced tremor (rat)	Acute; 12.5 – 25 mg/kg p.o.	↔ Tremor score [52]
Oxotremorine-induced tremor (mouse)	Acute; 25 – 50 mg/kg p.o.	↓ Tremor score [52]
<i>Lacrimation and salivation (mouse)</i>		
Oxotremorine-induced lacrimation and salivation	Acute; 3.13 – 200 mg/kg p.o.	↔ Salivation or lacrimation [52]
<i>Locomotor activity</i>		
Spontaneous locomotor activity (mouse)	Acute; 1 – 30 mg/kg i.p.	↔ Locomotor activity [35,63,64]
	Chronic; 10 mg/kg p.o. (twice daily, 28 days)	
	Chronic; 2 – 18 mg/kg s.c. (28 days)	
Spontaneous locomotor activity (rat)	Subacute; 40 mg/kg s.c.	↓ Locomotor activity [60]
Rotarod (rat)	Acute; 3 – 30 mg/kg i.p.	↔ Time on rotarod [24,62]
5-HTP-induced head movement (mouse)	Acute; 12.5 – 100 mg/kg p.o.	↑Head movements
5-HTP-induced head movement (rat)	Acute; 12.5 – 25 mg/kg p.o.	(dose-dependent) [52]
<i>Antidepressant activity</i>		
Forced swimming test (mouse)	Acute; 4 – 32 mg/kg i.p.	↓ Immobility time [52,56-58]
	Acute; 2.5 mg/kg i.p. + LY392098	
	Subacute; 25 – 100 mg/kg p.o.	
Forced swimming test (rat)	Acute; 5 mg/kg s.c.	↔ Immobility time [17]
	Acute; 5 mg/kg s.c. + WAY100,635	↓ Immobility time [17,59,60]
	Subacute; 10 – 40 mg/kg s.c.	
	Subacute; 40 mg/kg i.p.	
Tail suspension test (mouse)	Acute; 5 – 40 mg/kg i.p.	↓ Immobility time [61]
<i>Anxiety (mice)</i>		
Open field (mouse)	Chronic; 6 – 18 mg/kg s.c. (28 days)	↓ Time spent in center area [63]
Zero maze (mouse)	Acute; 3 – 30 mg/kg i.p.	↔ Time spent in open area [35]
	Chronic; 10 mg/kg p.o. (twice daily, 28 days)	↑Time spent in open area [35,64]
Stress-induced ultrasonic vocalization (rat)	Acute; 20 – 40 mg/kg i.p.	↓ USV duration (dose-dependent) [66]
Exploratory behavior in novel environment (mouse)	Acute; 2 – 40 mg/kg s.c.	↑Locomotor activity [65]

5-HTP: 5-hydroxytryptophan; 8-OHDPAT: (8-hydroxyl-2-(di-*n*-propylamino)tetralin; EEG: Electroencephalogram; i.p.: Intraperitoneally; mCPP: (*m*-chlorophenyl piperazine); p.o.: By oral gavage; REM: Rapid eye movement; s.c.: Subcutaneously.

Table 2. Published studies of duloxetine for preclinical behavioral tests (continued).

	Duloxetine dose	Effect
<i>Memory (rat)</i>		
Inhibitory avoidance task	Acute; 10 – 20 mg/kg i.p.	↔ Short- or long-term memory [67]
	Subacute; 10 – 20 mg/kg i.p. (5 days)	
	Acute; 10 – 20 mg/kg i.p. + MK-801	
<i>Sleep (rat)</i>		
Sleep EEG recording	Acute; 12.5 – 25 mg/kg p.o.	↓ REM sleep [52]
		↓ Slow-wave deep sleep [52]
		↑ Awake period [52]
	Acute; 7.7 mg/kg i.p.	↔ REM sleep [11]
		↔ Slow-wave deep sleep [11]
		↑ Awake period [11]
		↓ Paradoxical sleep [11]

5-HTTP: 5-hydroxytryptophan; 8-OHDPAT: (8-hydroxyl-2-(di-*n*-propylamino)tetralin; EEG: Electroencephalogram; i.p.: Intraperitoneally; mCPP: (*m*-chlorophenyl) piperazine; p.o.: By oral gavage; REM: Rapid eye movement; s.c.: Subcutaneously.

Anxiolytic effects of chronic duloxetine have been reported, as evident through an increase in the time spent in the open area in the zero maze test [35,64]. However, a decrease in the time spent in the central area in the open field test was also reported elsewhere, suggesting an anxiogenic effect [63]. Duloxetine also increases exploratory behavior in a novel environment [65] and it provokes a dose-dependent reduction in stress-induced ultrasonic vocalization [66], indicative of an anxiolytic-like effect.

No effects of duloxetine on short- or long-term memory have been reported in the rat inhibitory avoidance task [67]. Moreover, memory impairment induced by MK-801, an NMDA antagonist, is unaltered by duloxetine, that is, it is unable to recover the impairment on memory produced by this glutamatergic antagonist [67]. In summary, the effects of duloxetine most closely linked with depression are a reduction in body weight, food intake and, at higher doses, locomotor activity. Antidepressant-like effects of duloxetine have been demonstrated in rodents in the FST and TST, and anxiolytic-like effects in the zero maze.

4. Duloxetine and synaptic plasticity

In recent years, several findings have indicated the importance of neurotrophins and neuronal plasticity in mood regulation and antidepressant-like effects. The delay between the rapid increase in monoamine levels induced by antidepressants and the onset of clinical effects suggests that the antidepressant mode of action involves gradual alterations in synaptic plasticity. The neurotrophic hypothesis of depression postulates that low levels of neurotrophins underlie depression [68,69]. Neurotrophins are growth factors that are essential for the neuronal adaptations that mediate different aspects of mood regulation [68,70].

Several studies have investigated the effects of duloxetine on neurotrophin levels and neuronal plasticity. Chronic, but not

acute duloxetine treatment increases the expression of the mature form of brain-derived neurotrophic factor (BDNF) in both the cortex and hippocampus [50,71-73], and while the precursor form of BDNF (pro-BDNF) preferentially promotes programmed neuronal death, the mature form enhances neuronal survival and differentiation. Several other antidepressant drugs also augment BDNF, including those SSRIs and NRIs [74,75], implicating the upregulation of BDNF in the adaptive changes induced by antidepressants. Indeed, antidepressant treatment reverses the reduction in serum BDNF levels observed in depressed patients [76].

Arc (activity-regulated cytoskeleton-associated protein) is a growth factor and an immediate early gene that plays a fundamental role in neural plasticity, which has also been implicated in the modulation of mood [77,78]. An increase in Arc expression in rat cortex and midbrain after chronic duloxetine treatment [73] and in addition, a strong correlation between Arc and BDNF expression was observed in the cortex and midbrain after chronic but not acute duloxetine treatment. In the only study to investigate the effect of duloxetine on adult hippocampal neurogenesis, no changes were detected in cell survival or in the number of newly born cells in the dentate gyrus of the hippocampus.

Thus, together these findings indicate that adaptive changes in neuronal plasticity induced by duloxetine contribute to its therapeutic effect in the treatment of depression.

5. Other uses of duloxetine

Both 5-HT and NA monoamines modulate ascending spinal nociceptive neurotransmission via the descending inhibitory pain pathway [79,80]. Accordingly, although developed to manage depression, duloxetine may also produce analgesia in conditions of chronic pain [62,81-83] and indeed, it has been approved for the treatment of diabetic neuropathic pain [84].

Duloxetine is also effective in treating stress urinary incontinence due to the inhibition of 5-HT and NA reuptake induced in specific spinal cord motor neurons that innervate the striated muscle of the urethral sphincter [85,86].

6. Conclusion

Duloxetine belongs to a new group of antidepressants that selectively inhibit the reuptake of monoamines, particularly 5-HT and NA, showing little affinity for other receptors. As well as inhibiting the reuptake of monoamines, traditional TCAs have a high affinity for other receptor types, resulting in unwanted side effects. Recent findings have also demonstrated that duloxetine modulates the expression of neurotrophic factors, such as BDNF, which has important implications for our understanding of the etiology and evolution of depression, indicating that the basis of depression is more complex than simple monoamine deficit. Preclinical studies of novel drugs like duloxetine, which combine the blockage of monoamine reuptake with the modulation of neuroplasticity, provide significant insights into possible future approaches to treat depression. In addition, such studies highlight the potential of other similar compounds to treat disorders such as pain and cognitive decline, which are frequently associated with depression.

7. Expert opinion

Depression is one of the most prevalent mental disorders and it often represents a chronic condition. Furthermore, a large proportion of depressed patients respond poorly to the therapies currently available, underlining the need for new and innovative antidepressant drugs that are designed on the basis of our understanding of the molecular and biochemical processes that underlie this condition.

While several substrates have been implicated in the etiology of depression in recent years, the vast majority of the new antidepressants still target 5-HT and NA transmission. Duloxetine, a 5-HT and NA reuptake inhibitor, was introduced to the market some years ago, and has since proved an effective and well-tolerated antidepressant. Duloxetine inhibits 5-HT and NA reuptake both *in vitro* and *in vivo*, with greater affinity for the 5-HT than the NA transporter, and minimal effects on dopaminergic transmission. In this way, duloxetine presents low constant affinities for both monoamines transporters. However, compared with TCAs, duloxetine lacks relevant effects on neurotransmitter receptors, which conferred it the avoidance of the typical TCAs side effects. Additionally, duloxetine has a potential metabolite which would retain the ability for the inhibition of the reuptake of both monoamines.

In microdialysis studies, acute duloxetine increases 5-HT, NA and DA in the rat cerebral cortex in a dose-dependent manner, as

well as 5-HT and NA in the hippocampus and DA in nucleus accumbens. These findings demonstrate that duloxetine can modulate all three monoamines implicated in the symptoms of depression. By contrast, chronic duloxetine treatment has a significant effect on the autoregulation of NA and 5-HT release, altering the sensitivity of 5-HT_{1A} autoreceptors and α_2 -adrenoreceptors. These two autoreceptors are involved in monoamine release in terminal areas, which in turn modulates NA and 5-HT. Thus, desensitization of these autoreceptors accelerates the onset of any antidepressant effects.

Duloxetine is effective in most behavioral tests used to detect antidepressant-like activities. Moreover, this compound exhibits anxiolytic-like activity in several behavioral tests of anxiety in rodents, while displaying no effects on memory. On the other hand, duloxetine reduced food intake in rats showing low propensity to cause weight gain, TCAs typical side effect. Chronic duloxetine treatment increases BDNF expression in the cortex and hippocampus, which is strongly implicated in its antidepressant activity. Indeed, a large body of evidence suggests that BDNF-mediated neuroplasticity plays a pivotal role in both the pathophysiology and pharmacotherapy of depression.

Depression is characterized by a constellation of symptoms presented by the majority of patients. In addition to the so-called emotional symptoms, depressive patients may also experience physical symptoms, such as aches and pains. Importantly, through the same mechanisms that underlie its antidepressant effects (i.e., inhibition of 5-HT and NA reuptake), duloxetine exerts analgesic effects in several animal models of pain as other analgesic antidepressant drugs. However, duloxetine elicits its antidepressant and analgesic effects at the same clinical doses. This property might be useful in the comorbidity pain and depression condition or for treating physical painful symptoms of depression.

Together, the findings reviewed here demonstrate that the development and preclinical testing of duloxetine proves it to be an effective antidepressant for the long-term treatment of depressed patients.

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